Ciência Rural, Santa Maria, v.53:6, e20210801, 2023
http://doi.org/10.1590/0103-8478cr20210801
ISSNe 1678-4596
ANIMAL PRODUCTION

Performance evaluation of leg and ear numbers in radio frequency identification systems (RFID) in sensitive livestock products in goat breeding

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ABSTRACT: This study, evaluated the readability of electronic leg and ear tags in Saanen goats. Fifty-seven goats were identified with the electronic leg tags (ELT) and electronic ear tags (EET) from birth until the lactation period ends. Readability of ELT and EET was 96.30% and 90.55% respectively in static conditions at the end of 12 months. Foot and udder, with no infection rates for ELT and EET in calm and aggressive goats were 95.70% and 100%, respectively. No infection rates of foot and udder for ELT and EET in calm and aggressive goats were 95.70% and 100%, respectively. Tagging method and animal temperament was not statistically significant. As a result, low animal traceability with ear tags was determined by this study. Besides, it is suggested that smaller-sized tagging materials would be more accurate when the ankle was selected as a body area to place identification tags in goats. The resulting issue to be considered is that the leg tagging should not negatively affect the animal welfare and the foot and udder health. In the future, using a leg band in the identification of goats will become more widespread as it does not damage animals and has a high readability capacity.
Key words: animal welfare, goat, traceability, RFID, tags.

Avaliação de desempenho de números nas pernas e orelhas em sistemas de identificação por radiofrequência (RFID) em criação de caprinos

RESUMO: Este estudo teve como objetivo avaliar a identificação eletrônica nos membros e orelhas de cabras da raça Saanen. Cinquenta e sete cabras foram identificadas eletronicamente nas pernas (ELT) e nas orelhas (EET) desde o nascimento até o término do período de lactação. A leitura de ELT e EET foi de 96,30% e 90,55%, respectivamente, em condições estáticas ao final de 12 meses. As taxas de infecção de pé e úbere, em cabras calmas e agressivas, foram de 95,70% e 100% para ELT e EET em animais sem infecção, respectivamente. Não foi encontrado efeito significativo do método de marcação e temperamento animal. Com o resultado, a baixa rastreabilidade dos animais com marcas de orelha foi determinada por este estudo. Além disso, sugere-se que materiais de etiquetagem de menor porte seriam mais precisos quando a região do metatarso do animal fosse selecionado como área corporal para a colocação de etiquetas de identificação em cabras. A questão resultante a ser considerada é que a marcação da perna não deve afetar negativamente o bem-estar animal e a saúde dos pés e do úbere. Futuramente, o uso de etiquetas na identificação de cabras será mais difundido, pois não agride os animais e tem alta capacidade de leitura.
Palavras-chave: bem-estar animal, cabra, rastreabilidade, RFID, numeração.

INTRODUCTION

The traditional identification methods used in small ruminant production are notch, plastic, and tattoo. However, these methods are more painful for the animal then leg tagging. Besides, there is a significant loss of information due to the fall of the plastic numbers in traditional identification (ANON, 2006; PINNA et al., 2006). The proper identification of animals is essential. Proper identification enables the producer to keep records comprehensive. The efficient maintenance of this information requires a permanent identification system. Several systems of identification may be used. Plastic or visual ear numbering is a widely used method, but the method occasionally causes reading difficulties, or some of the printed numbers easily fall off (COOKE et al., 2010). These negative practices of plastic or visual numbers lead to difficulties in identifying the animals, especially in intensive enterprises. However, the electronic systems used in the identification or traceability of the animals facilitate the process (CURTIS, 2002) and provide more advantages than the traditional identification (notch, plastic, and tattoo) methods (CHING & TAI, 2009). In electronic identification systems, problems such as the inability

Received 11.08.21 Approved 07.21.22 Returned by the author 09.15.22
CR-2021-0801.R1
Editors: Rudi Weiblen Pasquale De Palo

to read the number or the animal information disturbances individually are less than the plastic numbering method (CARNÉ et al., 2010). Electronic rumen boluses do not adversely affect animal welfare and allow breeders to easily monitor their animals in addition to their usefulness during transportation (FONSECA et al., 1994).

The International Committee on Animal Registration (ICAR) has developed standards and guidelines for identifying animals. In recent years, both visual identification systems and electronic leg numbers have been developed in France and Spain, where more dairy goat farms are typical (HILPERT et al., 2009; TAŞKIN et al., 2016). Apparatuses used for the electronic identification of small ruminants have recently been taken under observation by many researchers. The advantage of the individual identification of animals, especially with their electronic leg numbers and rumen boluses, is their adherence to computer software that allows for better herd management based on the enterprises (MCCARTHY et al., 2009). Electronic Radio Frequency Identification (RFID) enables traceability systems to save labor and time and minimizes the margin of human error (Marina et al., 2020). According to the results of a survey study, the practical use of electronic identification systems was advised to the technicians and farmers (PINNA et al., 2006). In the study, it was particularly emphasized that the official animal identification systems currently used were not satisfactory and positive results with regard to the electronic RFID system were reported. However, there is a remarkable detail in this report. Identities worn as a foreign substance after they are inserted and in the future become more difficult to carry biologically and this cause infections. Every substance that will be wanted to be attached to the body will also carry the risk of infection. Risks increase in this similar procedures, especially in hot seasons when bacteria multiply easily. Another risk is experienced if the udders of milk-producing creatures are large. Breast infections will be inevitable when the electronic leg tags on the hind legs rub against the breast.

In this study, the performances of the electronic leg (ELT) and the electronic ear (EET) numbering methods in goats were simultaneously compared and their effects on the animal welfare and their applicability was examined.

MATERIALS AND METHODS

Animal material

Animal material of the study consisted of 57 female goats in their 2nd (15 goats), 3rd (14 goats), 4th (14 goats), and 5th (14 goats) lactation, and the data obtained during an entire lactation period (from January to October). The temperaments types assessment typically takes place as part of a routine weighing or handling procedure, such as a race or chute. Once the procedure is complete, the animal is released from the chute. The time it takes to cover a set distance along a raceway is calculated. This is called flight speed/time distance (HASKELL et al., 2014). The animal is released from the weighing machine. The time it takes to cover a set distance is calculated. This distance is typically short to capture the immediate response to release. When the goat is caught again immediately; if caught in a short time (less than 10 seconds), it was considered a calm goat. These goats usually stayed where they were and never tried to escape. Other goats fled away and were caught for long periods (over 10 seconds). These goats are defined as aggressive goats.

Study area

The study was conducted in Izmir province, which is a province that is located at the 38º north parallel and 27º east longitude in the Aegean Region, and 0-20 m elevation from sea level. The district is hot in the summer and warm and rainy in the winter. The moisture average is around 66%. The dominant winds are north and south. Due to the pressure differences during season changes, winds can blow in both directions, but they usually sweep south, south-west, and north in the winter.

Housing conditions

The research was carried out on the Saanen goats from the Sheep-Goat Enterprise of the Zoo Technology Department of the Faculty of Agriculture, Ege University, Izmir, Turkey. The enterprise has a pan having an open semi-intensive system with one side closed in the east-west direction and open on the other sides. Its roof is 2,5 m high, and it has inner departments separated by walls. This dairy goat shelter has the capacity for 60 animals and contains totaly of sections (96 m x4=384 m^2) and 12x2 milking sections equipped with electronic antennas in addition to its 4 separate kid growing departments (24 m x4=96 m^2). An automatic weighing machine measures the live weight of each goat after milking. In addition, the enterprise has one infirmary (24 m^2) and 12 kidding pens (3 $m^2 \times 12 = 36$ m^2). Inside the shelters are moorings with sequential locking mechanisms to the service road. In the combined feeders that were 120 cm high and 35 cm wide, roughage and concentrated feeds were mixed and given to the goats. In addition, the goats were given additional concentrated feed by the feed automat integrated with the RFID system according to their yield levels.

Herd management

The goats' feeding was programmed by evaluating them separately in three different periods. These periods were the early lactation period, the middle lactation period, and the last lactation (pregnancy) period the early lactation period, the goats were given crude and concentrated feed mixture containing 0,5 kg dry alfalfa, 0,3 kg wheat straw, 1,5 kg corn silage and 0,5 kg concentrated feed. In the middle lactation period, the mixture was arranged as 0,6 kg dry alfalfa, 0,2 kg wheat straw, 2,5 kg corn silage and 0,5 kg concentrated feed. The last lactation (pregnancy) period feeding mixture contained 0,4 kg dry alfalfa, 0,4 kg wheat straw, 1 kg corn silage, and 0,7 kg concentrated feed. The daily milk yields of the goats were controlled, and additional milk feed needs were determined, and they were given individually in the feed automat with an RFID system. After milking, data were collected twice a week from the goats that enter the weighing machine twice a week, from the weighing antenna. In addition, data were also collected from the goats that enter the automatic feeding section at least twice a day. Limb measurements in goats were measured just prior to the study before the tags were attached.

Administration and monitoring of identification apparatus

In the study, the data were collected from 57 head Saanen goats, 27 of which had electronic ear numbers and 30 of which had electronic leg numbers. The etiquette of the leg numbers was 181 x 39 mm in size and 22 mm in thickness with a weight of 21 g. In order to read the ear and leg numbers of the goats, one antenna took data while the goats were entering the milking section and during the weighing period and the other antenna took the data again. The data were taken from the goats when they entered the milking systems twice a day and this operation was repeated during the 270 days of lactation period. RFID, known as electronic identification, is based on reading using a transponder without a battery. Here is the reader activated by the signal sent by a reader. The code or the number on the ear that is read in response to the transmitted signal is detected. The energy requirement for the RFID device comes from the general electromagnetic field obtained from the reader. The absence of an internal energy source for the transponder limits its use period. The electronic number notified to official animal agencies operate with low radio frequency (134.2 kHz), and the International Standards Organization (ISO, 1996a, 1996b) regulates the code structure and the frontal surface.

Goats were kept with their kids in the pen for 3 days after birth. There was separate birth compartments for pregnant goats and new young kids and concrete water tanks in the barn. The barn was surrounded by a large, wood-fenced yard, and there were wooden feeders in the yard. All electronic tags were immediately attached to the goats that gave birth. All goats were identified on left ears with visual tags (Allflex) and the right ears with EET were used for compulsory official animal identification in Turkey. Electronic ear tags contained an FDX-B (full-duplex B) transponder, which worked at a frequency of 134,2 kHz, by following ISO standards (ISO, 1996a) (Figure 1). The button-button electronic EET weighted of 6,6 g and a diameter of 27,5 mm. The cost of one electronic ear tag per animal was 3 $. Electronic leg number etiquettes were (ELT) 21 g in weight 181 x 39 mm size at 22 mm thickness (Figure 2). The flag-type EET total weight was 4 g, and the dimensions for female and male pieces were 38x40 and 38x35 mm, respectively. The cost of one electronic leg tag per animal was 5 $. Both types of ear tags were tamper-proof, plastic, and white-yellow, and had a laser-printed unique identification number.

Retention and reading performance of ELT and EET were evaluated on-farm conditions for 1 year. Each EET was read under static conditions in restrained animals using a hand-held transceiver (reader) (Agrident APR500) with a built-in keyboard and integrated antenna. EET's dynamic reading efficiency was also evaluated by using an ISO-

Figure 1 - Electronic leg tag.

Figure 2 - Electronic ear tag.

compliant reader, which was connected to a 94x52 cm frame antenna installed on a plastic panel, which was able to read at a maximum distance of 100 cm for EET. The panel reader was mounted on the left side of a runway (width 50 cm). When the goats passed in front of the antenna, each electronic identifier was read, and the identification code from the panel reader was transmitted to the computer (CARNÉ et al., 2010).

Data processing

In the study, the following equations were used in the determination of readability (RE), retention rate [RR], and dynamic reading efficiency (DRE) trait in Saanen goats;

$$RE\ [\%]: \frac{n\ read\ devices}{n\ applied\ devices} \times 100\ (Caja\ et\ al., 2014) \quad (1)$$

$$RR\ [\%]: \frac{n\ retained\ devices}{n\ applied\ devices} \times 100\ (Caja\ et\ al., 2014) \quad (2)$$

$$DRE\ [\%]: \frac{number\ of\ numbers\ read\ from\ the\ transponder}{number\ of\ readable\ numbers} \times 100\ (Caja\ et\ al., 1999) \quad (3)$$

In Saanen goats, welfare measures are individually determined according to some defects, such as injuries, tears, or crushes, which could be caused by both the ear and the feet and the udder (Injury status: 1: Yes; 2: No; Injury place: 1: in the ear; 2: in the udder; 3: at leg).

Statistical analysis

In this study, the effects of the numbering (EET and ELT) methods on the examined characteristics were investigated (CAJA et al., 1999; ABECIA & TORRAS, 2009). Losses, electronic failures, and ear tags' readabilities were analysed in the Cox Proportional Hazard (COX, 1972) procedure. Non-significant effects (birth type, etc.) were removed from the model. A nonparametric Kaplan–Meier survival analysis and log-rank tests of equality across strata were performed for the ear tags in the LIFE-TEST procedure (CARNÉ et al., 2009a). All analyses were performed in SPSS (1999) Statistical Software (MEULMAN & HEİSER, 1999).

LIFETEST procedure of SAS permitted the comparison of the longitudinal readability of ID devices throughout the entire period of study without excluding right censored data (data from animals that left the study before a device failed), according to CANTOR (2003) and KLEINBAUM & KLEIN (2005), as well as the Kaplan-Meier estimates of readability for each type of device used.

RESULTS

In the study, average values were determined in terms of characteristics such as live weight, cannon circumference, ear length, and width generally favour the leg tag group. The average live weight in Saanen goats was 68,65 kg, cannon circumference was 11,27 cm, the ear length was 12.08 cm, and the ear width was 6,22cm. According to the identification method in Saanen goats, the descriptive statistics for some morphological characteristics were given in table 1.

In Saanen goats with EET, it was determined that the number of falling numbers from the etiquettes was relatively higher due to their temperament. In this numbering method, the readability ratio (RE) was also reported to be lower in aggressive animals than in calm animals. A similar situation was the case for the time spent to make the tagging. The average time spent on the application for calm animals was 15,6 seconds, and this value was 19,10 seconds for aggressive animals. The number of apparatus did not drop in ELT fitted goats. However, as in EET, the average time spent on animals was 14.9 seconds, and this value was 21,20 seconds in aggressive animals. The readability ratio (RE, %) was also reported to be higher in calm goats than in aggressive ones. The most important finding about to the number of falling numbers was that the numbers lost 6 times in EET group while there was not any decrease or loss observed in the ELT group. The identity information losses seen in the plastic tagging were not observed in the leg bands. This shows that there was a loss of $3 per animal when it was used in the electronic ear tag. This loss was calculated as $18 in total. In addition,

Table 1 - Descriptive statistics for some morphological characteristics.

	Characteristics	n	Min	Max	$\bar{X} \pm S_{\bar{X}}$
Electronic Leg Tag	Body weight	27	46	95	68.19 ± 2.53
	Cannon circumference	27	11	13	11.24 ± 0.12
	Ear length	27	11	13	12.00 ± 0.06
	Ear width	27	6	7	6.22 ± 0.07
Electronic Ear Tag	Body weight	27	47	84	69.07 ± 1.68
	Cannon circumference	30	11	13	11.30 ± 0.13
	Ear length	30	11	14	12.15 ± 0.11
	Ear width	30	6	7	6.23 ± 0.08
Overall	Body weight	30			68.65 ± 1.47
	Cannon circumference	57			11.27 ± 0.09
	Ear length	57			12.08 ± 0.07
	Ear width	57			6.22 ± 0.05

the readability of electronic systems was lower (81,36%) in electronic tags attached to the head limbs due to the small area of the goat's leg. ELT attached to the shins of the leg was more readable (89,99%). The identification method in the Saanen goats and the readable findings, according to the data, were given in table 2.

The rate of any infection observed in goats is generally 4,3 %, which is like 0.07 % in animals with leg infections. The values determined for udder infections were similar (0,04 %) to the infections occurring in the animal's leg. No ear infection has been observed in calm-tempered Saanen goats. The incidence of ear infections was 25 % in animals with aggressive structures, and no infection was detected in the udder and leg. The variance of some well-being measures was given in table 3 according to the method of tagging and the temperament of the goats.

DISCUSSION

One EET, and the other ELT effect were examined, and the compatibility of these results with the literature was examined in goats. Unlike ear tags and ruminal boluses, leg tags are secured permanently to the animal now of application. Therefore, the tag should allow the operator to leave some space between the leg and the tag so that it is

Table 2 - Comparison with electronic identification methods.

	---------------Electronic Ear Tag (n=30)------------		-------------Electronic Leg Tag (n=27)-----------	
	--------------------Temperament type----------------		------------------Temperament type----------------	
Characteristics	Calm	Aggressive	Calm	Aggressive
Number of tags	12	18	11	16
Time spent for tagging (sec)	15.60	19.10	14.09	21.20
Number of losing numbers	1	5	-	-
Number of unreadable numbers	36	30	2	8
RR (Readability rate, %)	96.66	83.33	92.59	70.33
Chi-Square P Value:	-------------------------0.554-------------------------		------------------------0.436*------------------------	

Table 3 - Change of some parameters of welfare according to the goat temperaments.

			----Foot Infection----			--Udder Infection--			----Ear Infection----		
Temperament	Method	Statistics	No	Yes	Total	No	Yes	Total	No	Yes	Total
Calm	ELT	n Rate of tagging method (%) Rate of foot/udder infection (%)	22 95.7 45.8	1 4.3 100.0	23 100.0 46.9	22 95.7 45.8	1 4.3 54.2	23 100.0 100.0	23 100.0 46.9	- - -	23 100.0 46.9
	EET	n Rate of tagging method (%) Rate of foot/udder infection (%)	26 100.0 54.2	- - -	26 100.0 54.2	26 100.0 54.2	- - -	26 100.0 54.1	26 100.0 53.1	- - -	26 100.0 53.1
Aggressive	ELT	n Rate of tagging method (%) Rate of foot/udder infection (%)	4 100.0 50.0	- - -	4 100.0 50.0	4 100.0 50.0	- - -	4 100.0 50.0	4 100.0 57.1	- - -	4 100.0 57.1
	EET	n Rate of tagging method (%) Rate of foot/udder infection (%)	4 100.0 50.0	- - -	4 100.0 50.0	4 100.0 50.0	- - -	4 100.0 50.0	3 75.0 42.9	1 25.0 57.1	4 100.0 100.0
	Chi-Square P Value:			0.469*			0.470*			0.531	

ELT: Electronic leg tagging.
EET: Electronic ear taggi.

not constraining but does not allow the tag to be lost. Some types of electronic leg tags allow an increase in leg diameter as the animal grows but can be difficult to affix to the animal and can be tampered with.

Evaluation of readability

According to the results, the readability of electronic systems were lower (81,36%) in electronic tags attached to the head limbs due to the small area of the goat's leg. ELT attached to the shins of the leg was reported to be more readable (89,99%). In electronic identification systems, the probability that the number cannot be read or mixed is a lower probability than in a traditional tagging device. There may be confusion during the reading of plastic or visual numbers (CAPOTE et al., 2005). This risk is lower in electronic identification systems because the probability of the device losing/disappearing is low, and more than one animal can be identified simultaneously (AIT-SAIDI et al., 2008a; 2008b). Published accounts of the effect of a farm system on retention rates of external identification tags are limited. However, it has been reported that the type of fencing influenced ear tag losses and failures in pigs; tag losses were reduced after stone blocks replaced barbed-wire fences (GOSÁLVEZ et al., 2007). Iberian pigs were significantly less likely to lose electronic ear tags when they were in enclosures with a stone-wall rather than a grid wall perimeter (JAUME et al., 2012). A comprehensive report by the Canadian Cattle Identification Agency, where several visual ear tags were compared in differing Canadian environments (brush, grass, forest, native grass, rocky), concluded that environmental conditions and on-farm management (feeder design, fence design). That should be considered when choosing tags that will meet minimum tamper-evidence, retention and readability rates (STANFORD et al., 2001).

Time management

The average time spent on the goats was 17,64 seconds for the ELT and 7.35 seconds for the EET. This timing was higher than the application of the EET rumen bolus (CAPOTE et al., 2005; KOWALSKO et al., 2014). According to CARNÉ et al. (2010), the use of rumen bolus in the study determined 22 seconds. The time spent applying leg tags per individual animal in the present study was less than the time reported for applying 'Animal comfort' leg tags (53 s) (CARNÉ et al., 2010), but was like the times required to apply an electronic bolus in several breeds in the United States of America (22 s) (CARNÉ et al., 2009a) and in kids and adult goats (28 s), or the time required to introduce injectable transponders in lambs and kids (30 s to 40 s) (CARNÉ et al., 2009b; ABECİA et al., 2004). In our study, the reason for the high duration of application is that it can be caused by stress in animals (QUEOROGA et al., 1994). In other words, less stress and good animal welfare are essential factors in decreasing application time (CAJA et al., 2005).

Experienced loss

In this section, ELT and EET evaluate loss performance. When we examine what was reported in some of the field studies in France, the study results showed that the ELT drop rate in the first studies in goats were 12,9 %, depending on the herd management on the farms. Losses, with models developed 0-5,6 % of the variation, but have reached values lower than 2,6 % (CAJA et al., 2004; BALWAY et al., 2010). In the case of hand-held readers, the readability rate varies between 65,5 and 92,3% due to loss in farm conditions. However, there is also an unreadable ELT. When the reason for this was investigated, it was determined that the breakage of the numbers and water and mud. In the use of ELT, ABECIA & TORRAS (2009) in Spain performed a study of the age range and live weight. As a result of the study, it was determined that the earliest age of wearing the leg number was 6 months and that in goats, ELT could be used to identify permanently without causing any negativity. GHIRARDI et al. (2006) reported that the loss/fall rate in plastic or visual ear number was 3.3 % in sheep. CARNÉ et al. (2010), in a study carried out on dairy goats, ELT, and EET in this rate, respectively, 4.3 % and 3.3 %. These values are well above the values reported by ICAR. THURNER & WENDL (2007) reported that ELT fall's overall rate was 9.9 % for sheep and 19.2 % for lambs at approximately 3 months old. These values are higher than the results of this study (0.00%). It was thought that good flock management (feeding, housing, etc.) on the research farm might have caused this situation.

Animal health and welfare

The large or small ear structure of the Saanen goats, which is the research material, is closely related to the more frequent injury/bleeding occurrence in the ear. There is no statistically significant difference in the number of dropping numbers in large-ear animals. In the evaluation made six months after the application of goats with large earrings, the number of not dropping is 95,5 %; therefore, the large ear numbers are not recommended by ICAR in France (CASTRO et al., 2004; GARÍN et al., 2003; GARIN et al., 2005; AIT-SAIDI et al., 2013). It has been reported that tissue damage is caused by rupture, bleeding, inflammation, and application in sheep-goat ears. Similar reduction rates were obtained in animals with small ear and rumen bolus (TORRAS et al., 2006). Due to the heavy amount of friction/avoidance behaviours observed in goats, large ear numbers be seen to decrease from time to time. This behaviour; barbed wire or hard surfaces are formed by friction. Because of this behaviour, either a tear in the ear or a drop in the ear number occurs (KARAKUS et al., 2015; KARAKUŞ & KARAKUŞ, 2017). In other words, the size of the ear number and the environment in which the animal is located directly affect the decrease of the numbers (OCAK et al., 2013).

CONCLUSION

As a result, it can be said that ICAR (2007) has been widely used in EU countries since the introduction of electronic identification methods; however, there has been a significant loss of information in many companies in non-member countries like Turkey. This situation makes it necessary to record breeding and breeding awareness in the regions and countries where regional or national breeding programs are implemented (ICAR, 2012; 2014). In animal husbandry enterprises, whether the production system is intensive, semi-intensive, or extensive is the decisive factor in deciding the electronic numbering method. The cost of the method chosen is another crucial issue in terms of animal welfare and food safety (ERMETIN, 2021). Otherwise, sustainable small ruminant breeding cannot be mentioned. The most critical issue to be considered is the minimization of the inability to read the electronic numbers attached to animals (SILVA et al., 2022). The tagging of an electronic leg has become more preferred in developed countries because of the loss of numbers or low fall rates in goats. However, it is suggested that smaller-sized materials would be more accurate when the ankle was selected as a body area in goats. Another essential issue to be considered is that numbering should not cause any adverse effects, primarily on animal welfare and leg and udder health. Finally, electronic leg and ear tags demonstrated similar on-farm efficiency for the identification of Saanen goats. They fulfilled the minimum 90.5 % efficiency required by NQ (Number questioning by the Ministry of Agriculture) for an official animal identification device at the end of the first year after tagging. However, studies determining the effects of electronic identification devices on animal traceability should be conducted on larger numbers of animals in different rearing systems in Turkey.

ACKNOWLEDGMENTS

This study was supported by Ege University Scientific Research Projects Directorate under the Grant 2015 ZRF 060: A Research on Determination of Use Goats on Performance of Electronic Leg and Ear Tag, 2015-2018.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

Permission of the ethics committee regarding the animal experiments was received for this study (Protocol Number: 2015-051, Experimental Animal Research Ethics Committee). All procedures performed in this study followed the ethical principles of the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

ÇK, TT and NK conceived and designed experiments. ÇK, CB and TT performed the experiments, CB carried out the lab analyses. TT supervised and coordinated the animal experiments and provided clinical data. TT performed statistical analyses of experimental data. ÇK, CB, TT and BAT prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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